Notes on some Difflugiidae from Norfolk (Rhizopodea, Protozoa)

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Introduction

The first British records of Rhizopoda and Heliozoa were contained in a privately-printed volume (Brightwell, 1848). The book comprised records and drawings of infusoria found by Brightwell in Norfolk, amongst which was the description of a species of *Difflugia*, tentatively identified as *D. globulosa* Dujardin. The only other records of *Difflugia* in the eastern counties of England are contained in Cash & Hopkinson (1909), otherwise the region has been neglected. The present paper is part of a series dealing with species belonging to the genus *Difflugia*, and is concerned with spherical or ovoid specimens which were isolated from a sample collected at Woodbastwick Fen, Norfolk. Three species of *Difflugia* were abundant in the sample, namely, *D. gramen* Penard, 1902; *D. lobostoma* Leidy, 1879 and *D. tuberculata* (Wallich, 1864) whilst two, *D. achlora* (Penard, 1902) and *D. wailsei* nom. nov. were represented by only a few specimens. The opportunity has also been taken to describe another member of the family Difflugiidae, that is *Cucurbitella mespiliformis* Penard, 1902, which was present in the same sample.

Materials and methods

A sample of algae and water plants was collected from a drainage dyke adjoining a reed marsh, at Woodbastwick Fen, Wroxham, Norfolk, in August, 1979. Specimens were extracted using a glass micropipette, washed in distilled water, and prepared for scanning electron microscopy using the techniques described by Ogden (1979*a*). Prepared stubs were examined on a Cambridge Steroscan S180 operating at 10kV and the results recorded on Ilford HP5 film.

Results

Difflugia gramen Penard, 1902

The shell is transparent, sometimes light brown in colour, spherical but tapering slightly near the aperture (Fig. 1). Although the general outline is usually constant, the texture of the shell varies between rough and smooth which may occasionally distort the outline. It is composed of a mixture of small to medium pieces of quartz, so arranged to make it intermediate in strength between fragile and robust. The particles are bound together by organic cement, small areas of which are frequently seen as part of the shell surface (Fig. 6). The surface of the cement is made of a network with a connecting membrane between the mesh (Fig. 4). The aperture is trilobed and bordered by an irregular raised ridge of small particles which are cemented together (Figs 2 & 3). A ring of small pores, about ten or more in number, surround the aperture slightly below the border of small particles (Figs 3 & 5). These pores are distinct openings in the organic matrix of the shell wall, but are sometimes covered by a thin smooth membrane.

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The measurements for individual specimens are shown in Figure 7 and the range is given in Table 1. The measurement for the diameter of the aperture is taken as the internal distance between any two lobes, as indicated in Figure 2.

Although *D. gramen* has been the subject of two recent works (Stepánek & Jiri, 1958; Gauthier-Lièvre & Thomas, 1958) the difference between this species and three similar species, namely *D. limnetica* (Levander, 1900), *D. lobostoma* Leidy, 1879 and *D. achlora* (Penard, 1902), have not been adequately explained. Penard (1902) listed the differences between these four species and separated them mainly on size, colour and the presence or absence of an apertural collar. The specimens described here are in good agreement with the original description of *D. gramen*, but are somewhat longer than previously quoted measurements. For example, Penard (1902) and Gauthier-Lièvre & Thomas (1958) examined specimens ranging from 60–80 μm, whilst Stepánek & Jiri (1958) and Vucetich (1973) gave measurements between 71–92 μm. Variation of the shape of the apertural lobes appears to be common and several examples are illustrated by Stepánek & Jiri (1958).

Table 1 Range of measurements (in μm)

	Length (L)	Breadth (B)	Diameter of aperture (Da)	B/L	Da/L
D. achlora n=2	49–54 (52–69)	43–46 (41–53)	16–17 (14–25)		
D. gramen n=35	89–117 (71–92)	70–112 (53–65)	23–39 (17–28)	0·96 ± 0·07	0·34 ± 0·04
D. lobostoma n=44	123–186	103–166	32–60	0·87 ± 0·06	0·29 ± 0·03
D. tuberculata n=27	102–152	88–141	29–44	0·89 ± 0·10	0·29 ± 0·04
D. wailesi n=6	96–112	79–90	29–34	0·80 ± 0·03	0·30 ± 0·01

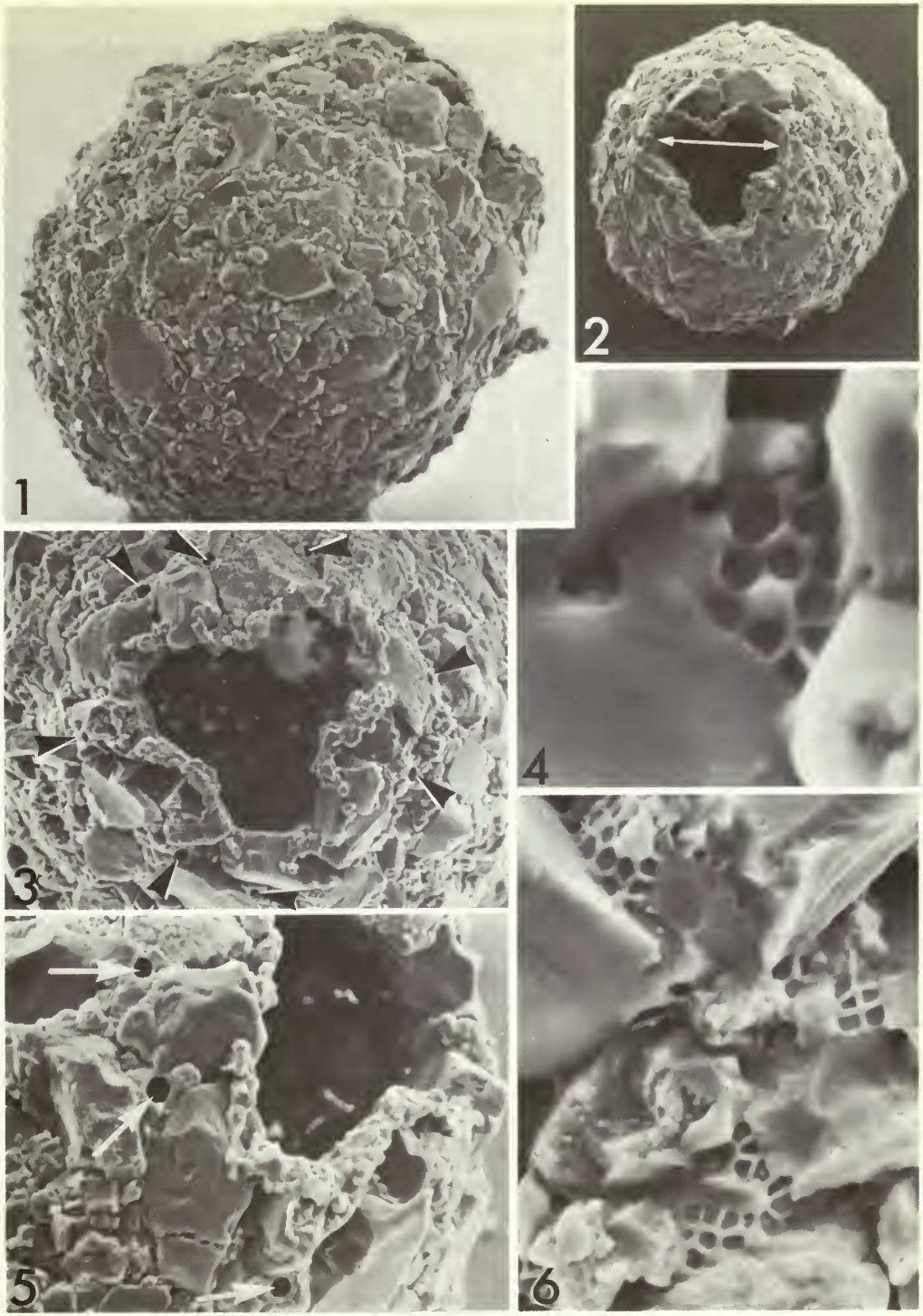
n= number of specimens measured
Bracketed measurements are taken from Stepánek & Jiri (1958).

Specimens of these species identified by Penard, in the Collections of the Department of Zoology, British Museum (Natural History), were examined for an apertural collar. It would appear that in lateral view specimens of *D. limentica* may have a small collar, whereas *D. gramen* and *D. lobostoma* have indications of some structure which could be considered to represent a collar. Unfortunately most of the specimens of these species are in lateral positions, as permanent preparations on slides, so that observations on the apertural opening are not possible.

Difflugia lobostoma Leidy, 1879

The shell is transparent, sometimes brownish in colour, ovoid or subspherical, and circular in cross section (Figs 8 & 9). It usually has a comparatively smooth outline, is robust and composed of a mixture of small to medium pieces of quartz. The quartz is bound together by organic cement which has the same structural network (Fig. 11) as that seen in *D. gramen* (Fig. 4). The aperture is usually trilobed and bordered by small particles (Fig. 10). Variation of the apertural opening ranges from squarish to curved lobes, the latter similar to those illustrated (Fig. 2) for *D. gramen*, and infrequently the aperture appears to be either four-lobed or as an irregular opening with indistinct lobes.

The measurements for individual specimens are shown in Figure 7 and summarized in Table 1.



Figs 1–6 *Difflugia gramen* Fig. 1 Lateral view ×870; Fig. 2 View of aperture to show the irregular ridge (The arrows indicate the distance measured for the diameter of the aperture) ×460; Fig. 3 Detail of aperture to indicate the ring of pores (arrowheads) × 1000; Fig. 4 Detail of organic cement network ×13000; Fig. 5 Side view of aperture to illustrate position of pores (white arrows) in relation to the apertural ridge ×1450; Fig. 6 Part of shell surface to show distribution of organic cement ×5300.

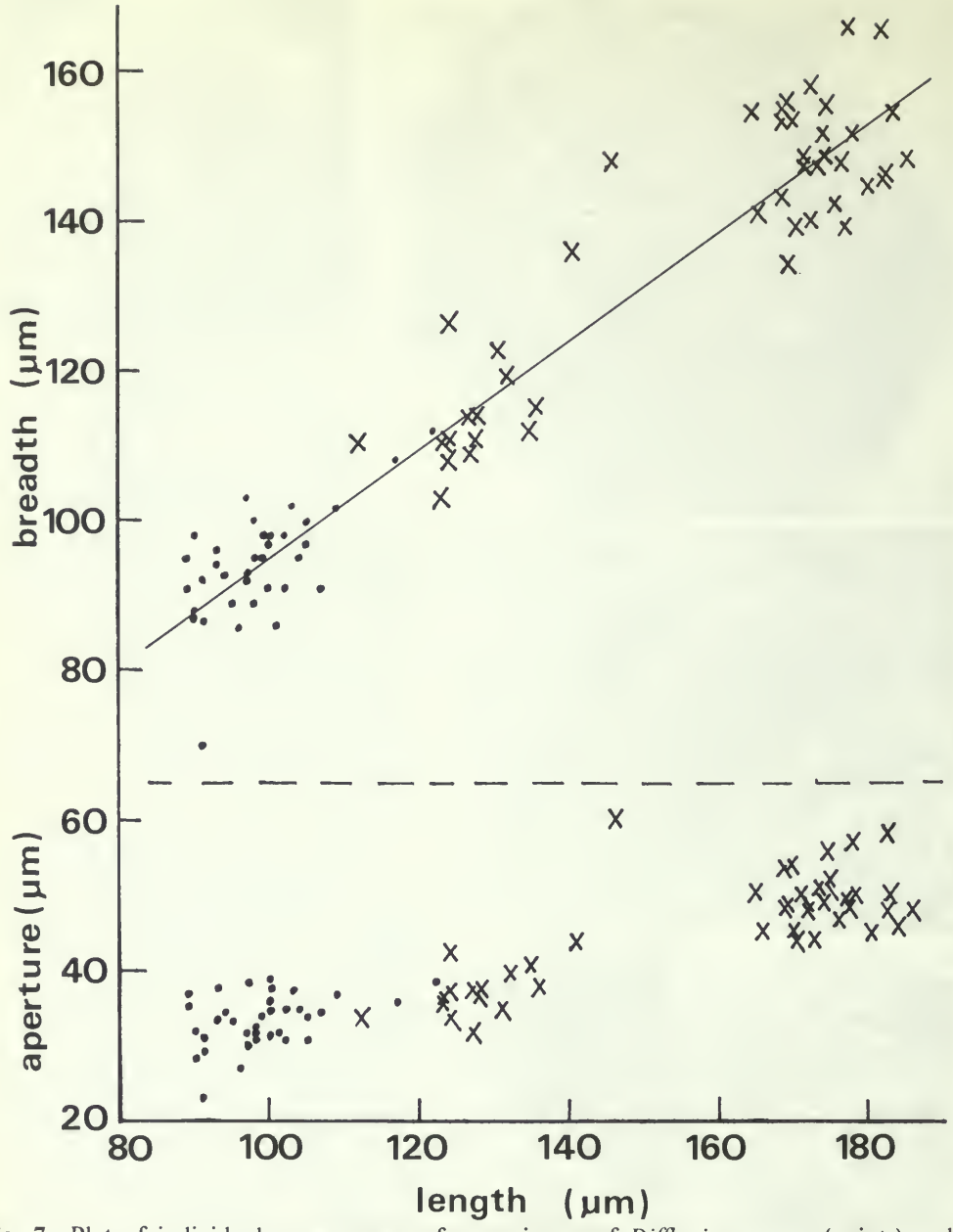
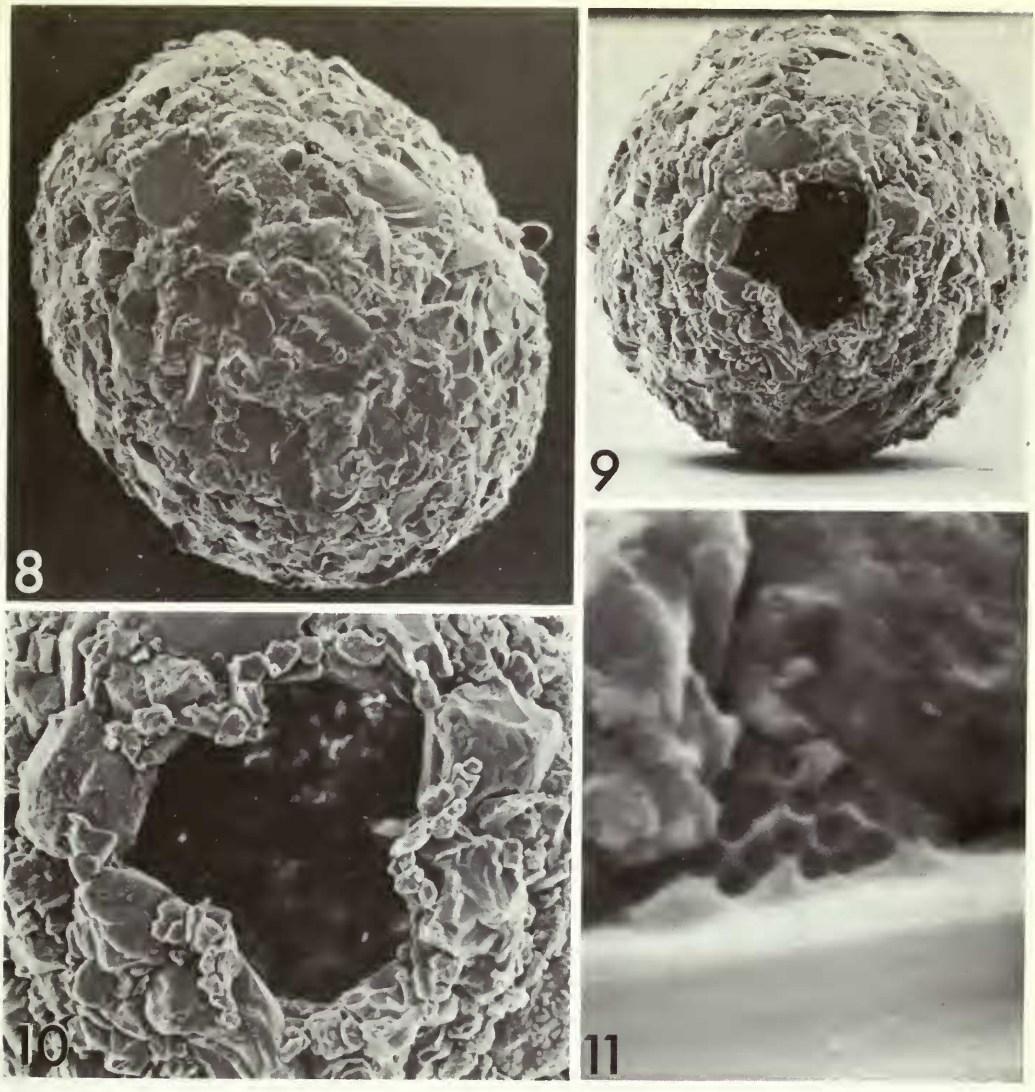


Fig. 7 Plot of individual measurements for specimens of *Difflugia gramen* (points) and *D. lobostoma* (crosses)—breadth (upper part) and diameter of aperture (lower part), vertical axis; total shell length, horizontal axis.

In the general description of *D. lobostoma*, Leidy (1879) described the shell as being usually composed of quartz-sand and rarely of diatoms or chitinoid material, whilst the aperture was usually from three- to six-lobed. His illustrations are so precise that we can identify at least five distinct species (*Difflugia achlora*, *D. gramen*, *D. tuberculata*, *Netzelia oviformis* (Cash, 1909) and *Cucurbitella mespiliformis*) amongst those that he attributed to represent *D. lobostoma*. Wailes (1912) identified some of the illustrations given by Leidy which at that time had been given specific names, but lists only *D. gramen* and *D.*



Figs 8–11 *Difflugia lobostoma* Fig. 8 Lateral view ×420; Fig. 9 Apertural view ×390; Fig. 10. Detail of aperture to show incomplete ridge of small particles ×900; Fig. 11 Portion of shell surface to show organic cement matrix ×10000.

tuberculata from the description of *D. lobostoma*. Unfortunately, the original description has not been amended to exclude those features that have since been considered to be the diagnostic characters of other named species, even though in his discussion Leidy (1879) indicated the most common features and measurements for *D. lobostoma*. The opportunity is taken here to redefine *D. lobostoma* from the original description (Leidy, 1879) and to designate the type figures: *D. lobostoma*—shell usually ovoid, composed of angular quartz-sand, with a trilobed aperture, fundus rounded, about 120–140 μm long and 100–128 μm broad, Figs 1-4, Pl. XV (Leidy, 1879).

Penard's (1902) description of *D. lobostoma* agrees well with the original differing slightly in size, 140–170μm, and in the shape of the aperture which was stated to be four-lobed in the form of a cross, although the cross frequently had an irregular outline. The descriptions given by Cash & Hopkinson (1909) and Vucetich (1973) are in general agreement with both Leidy

and Penard, but their specimens range in size from 90–115 μm and 65–110 μm respectively. The first authors, further suggest that the sinuous nature of the three to six-lobed aperture could be useful in separating these ovoid forms. Gauthier-Lièvre & Thomas (1958) have used the shape of the aperture to describe new varietys and forms.

Difflugia achlora (Penard, 1902)

Although only two whole and one broken specimen of this fragile species were found, it was considered desirable to describe them because of their contrasting shell structures. Both of the complete specimens have an elongated ovoid shell and an irregular trilobed aperture. The first is composed of a mixture of small pieces of quartz (Figs 12 & 13) and the second of a mixture of small diatom frustules and fragments of these siliceous structures (Figs 14 & 15). The organic cement that binds the shell components together, in both cases, is in the form of a network (Figs 16 & 17). The diameter of the mesh is about 0·02 μm, which is about half the diameter of the mesh of both *D. gramen* and *D. lobostoma.* A few small particles are seen surrounding the apertural opening, and a few pores are present in the shell just posterior to the aperture. The measurements are given in Table 1.

The specimens described here are similar to those described by Penard (1902), except that neither of the present specimens have a collar, which was a diagnostic character of the original description. Penard described the casing as yellowish with brown veins dotted amongst the overlapping plates, these veins were later thought to be very small beads by Decloitre (1948), and it would appear that they were both probably commenting on the organic matrix of the shell.

Difflugia tuberculata (Wallich, 1864)

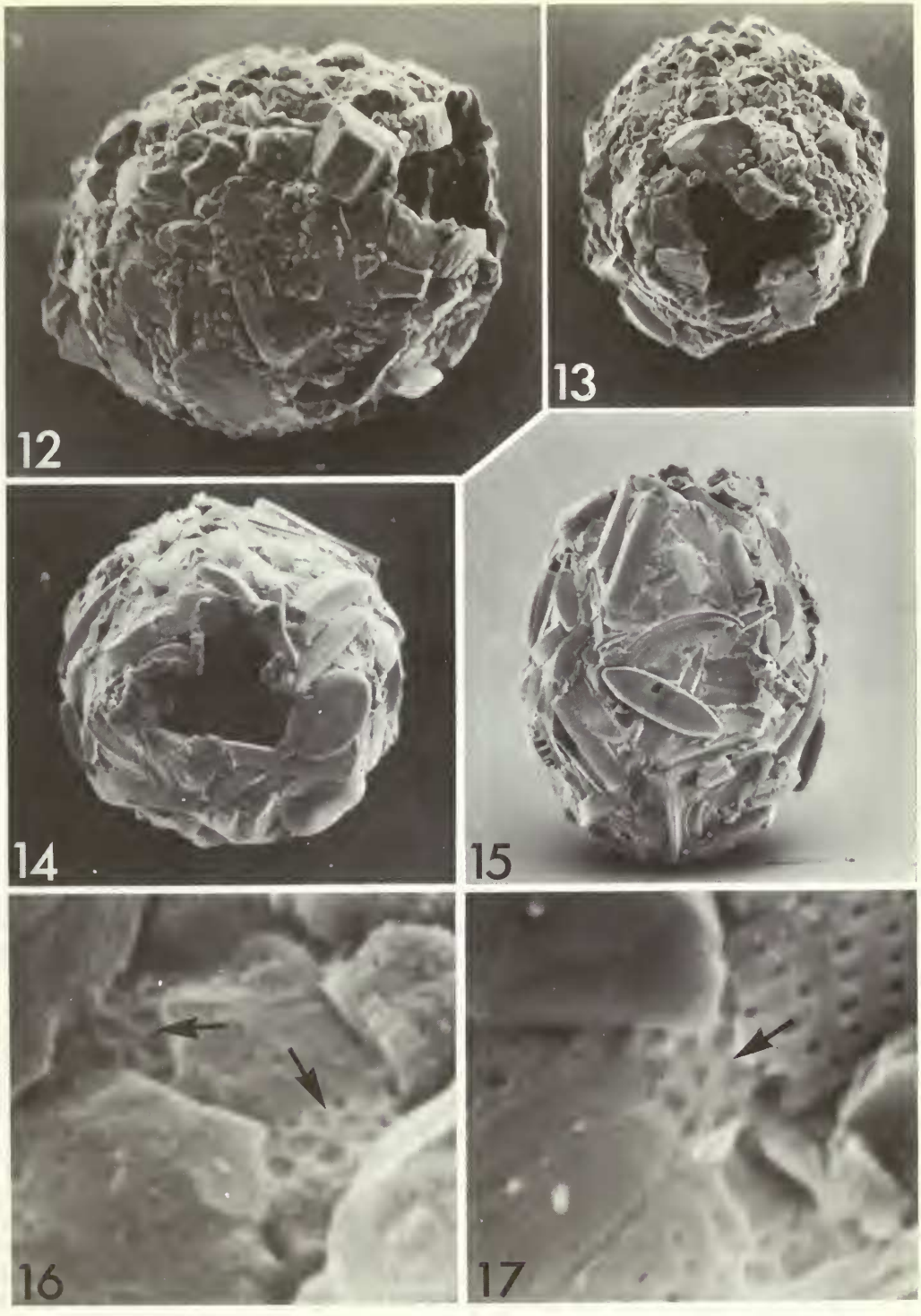
Our previous description (Ogden & Hedley, 1980) of this species was based on specimens whose shells were composed mainly of quartz particles. The present specimens have shells that are made mainly of diatom frustules, small siliceous elements and a few quartz particles. The outline is characterized by typical protuberances or bosses (Figs 18 & 19), whilst an abundance of organic cement in the form of a network, binds the shell particles together (Fig. 19). About ten small pores in the cement matrix, are arranged in a ring on the shoulder of the shell surrounding the aperture (Figs 21 & 22). A narrow projecting collar composed of small siliceous elements, surrounds the roughly circular aperture (Fig. 22). This collar is irregular and gives the apertural opening either an hexagonal or heptagonal appearance (Fig. 21), often these divisions are pointed or tooth-like (Fig. 20).

Penard (1902) noted specimens which he thought were polymorphic or transitional stages of *D. tuberculata*, and divided these into five groups on the basis of variation in the shape of the aperture and their different shell surfaces. Comments on variation of shell structure, from the present study, are included in the general discussion (see p. 135). Recently observations (Ogden, 1979*b*) on a few specimens from the Everglades, U.S.A., led me to suggest that the description of *D. tuberculata* might have to be amended to include specimens whose shells were composed mainly of diatom frustules. The present work has facilitated this emendment.

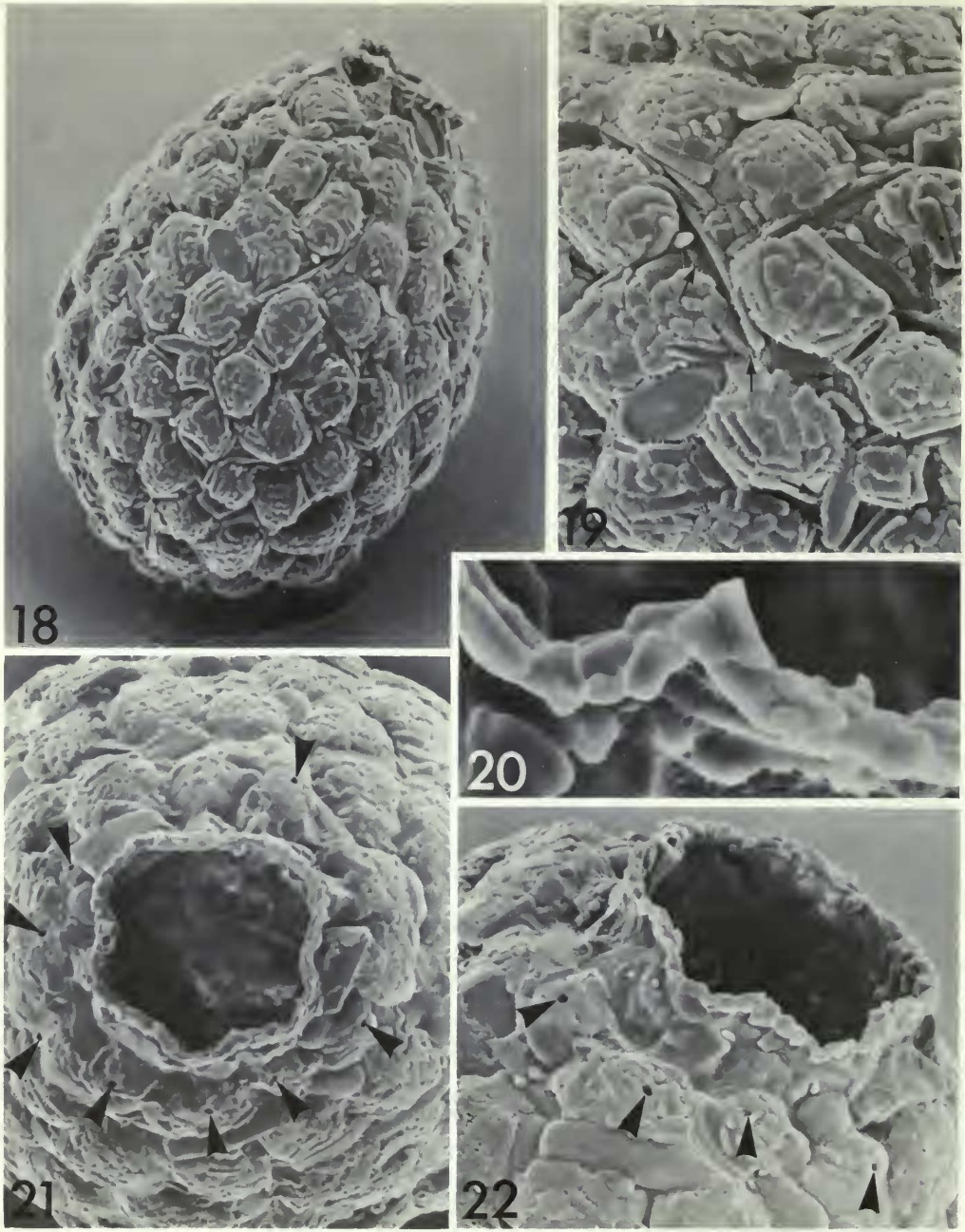
Difflugia wailesi nom. nov.

syn. *D. tuberculata* var. *minor* Wailes, 1919

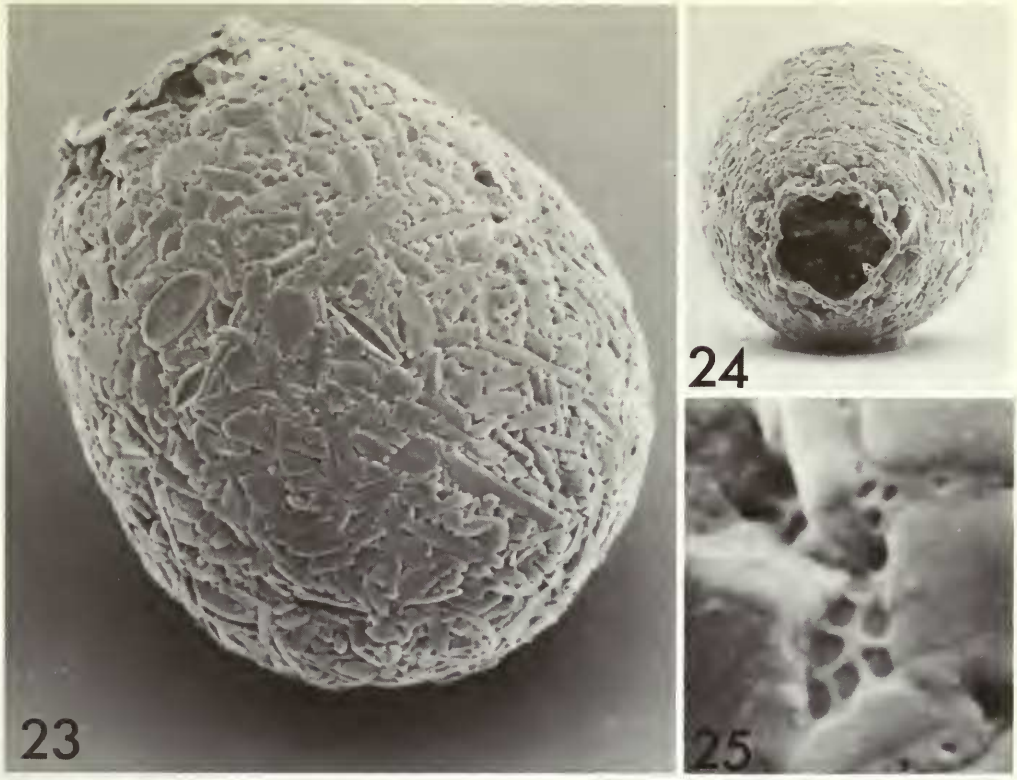
The shell is transparent, colourless, ovoid tapering towards the aperture (Fig. 23). It is fragile, has a smooth outline and is composed of a mixture of mainly diatom frustules and some flattened particles of quartz, bound together by a network of organic cement (Fig. 25). Just posterior to the aperture is a ring of small openings in the organic cement matrix, similar to those illustrated for *D. tuberculata* (see Fig. 21). The aperture is polygonal with either four or five indentations (Fig. 24) and is bordered by a slightly raised lip made of small particles cemented together.



Figs 12–17 *Difflugia achlora* Fig. 12 Lateral view of 'quartz' specimen ×1300; Fig. 13 Apertural view of 'quartz' specimen ×1100; Fig. 14 Apertural view of 'diatom' specimen ×1100; Fig. 15 Lateral view of 'diatom' specimen ×950; Fig. 16 Detail of organic cement ('quartz') ×12500; Fig. 17 Detail of organic cement ('diatom') ×14000.



Figs 18–22 *Difflugia tuberculata* Fig. 18 Lateral view to show typical protuberances ×580; Fig. 19 Detail of protuberances to indicate areas of organic cement (small arrows) ×1100; Fig. 20 Part of ridge that surrounds the aperture ×4100; Fig. 21 View of aperture to illustrate the ring of pores (arrowheads); Fig. 22 Side view of aperture to show position of pores (arrowheads) on shoulder of shell ×1200.

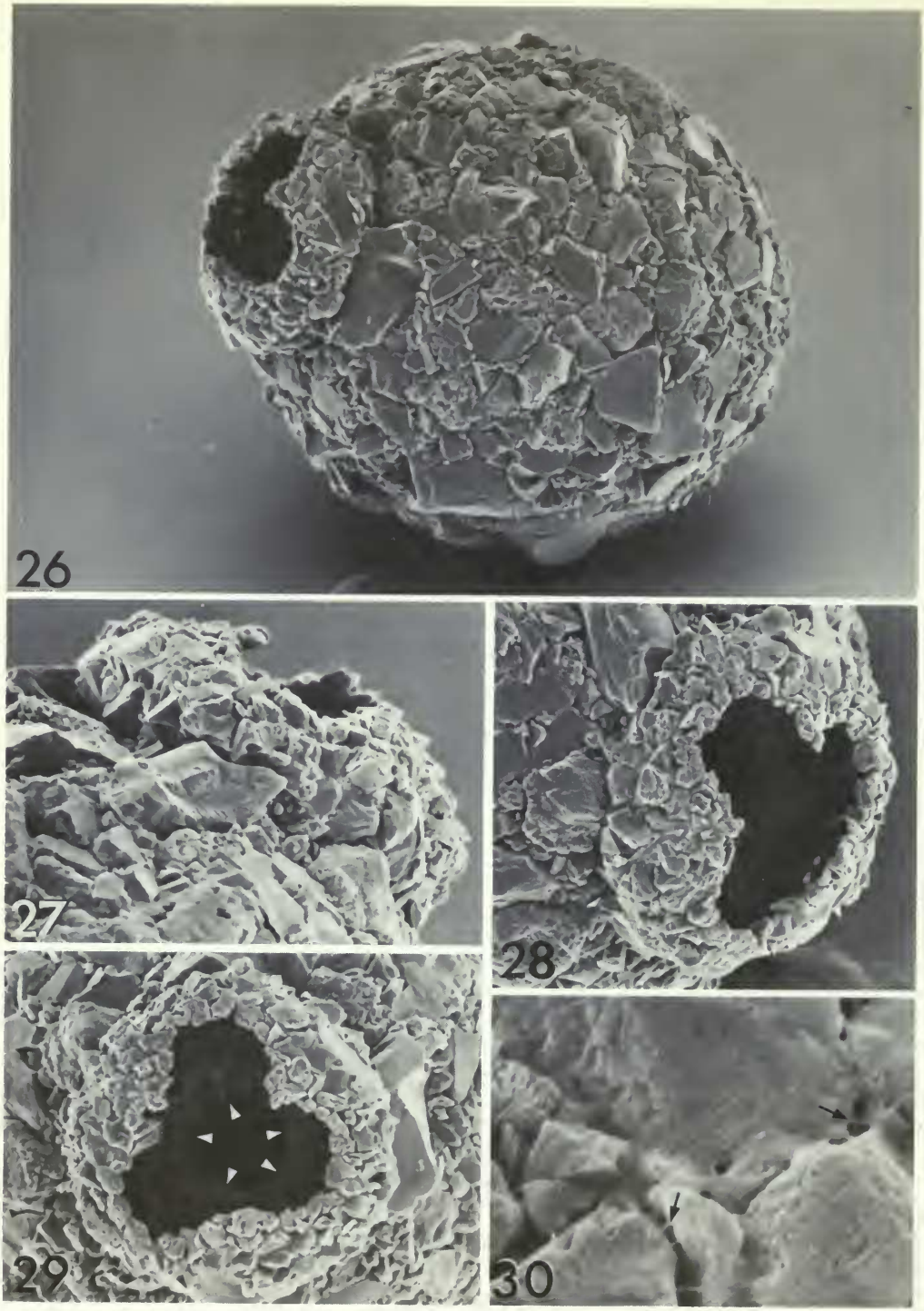


Figs 23–25 *Difflugia wailesi* Fig. 23 Lateral view to show smooth shell surface ×830; Fig. 24 Apertural view ×460 Fig. 25 Detail of organic cement network ×7700.

Penard (1902) described a small form of *D. tuberculata* which had a smooth shell. Specimens identical to these were listed as *D. tuberculata* var. *minor* by Wailes (1912), although the description of this variety appeared much later in Cash *et al.* (1919). In this latter work, Wailes refrained from describing these specimens as a distinct species because of Penard's (1902) earlier report regarding intermediate forms of *D. tuberculata*. More recently it was considered (Ogden, 1979*b*) that Penard's smooth specimens were possibly referable to the genus *Netzelia* Ogden, 1979. Confirmation of the correct generic status of this species must await evidence to show whether it can produce autogenous siliceous shell components, a prerequisite for the genus *Netzelia*, but this information can only be obtained from observations on this animal in culture. Until such information is available these smooth shelled specimens are here raised to species level in the genus *Difflugia*.

Cucurbitella mespiliformis Penard, 1902

The shell is dark grey or opaque, ovoid with a pronounced annular collar at the apertural extremity (Figs 26 & 27). It is composed of a mixture of small to medium pieces of quartz blended together to produce a strong shell with a relatively smooth outline. The organic cement that binds the particles is not very evident, but is occasionally seen at junctions (Fig. 30). The collar that surrounds the aperture usually has a regular three or four-lobed opening, but it may sometimes have a distorted outline, and is composed mainly of small pieces of quartz (Fig. 28). The divisions between the lobes may often appear as sharply pointed projections (Figs 28 & 29), due to the arrangement of small particles bordering the lobes. The aperture is a small roughly circular opening, on a level with the shoulders of the main shell



Figs 26–30 *Cucurbitella mespiliformis* Fig. 26 Lateral view to show apertural collar ×680; Fig. 27 Lateral view of apertural collar ×1200; Fig. 28 Latero-apertural view of apertural collar to illustrate the sharply defined projection dividing two lobes ×1100; Fig. 29 View of apertural collar to show the inner aperture opening (small arrowheads) ×1100; Fig. 30 Detail of shell surface, the small amounts of organic cement visible are arrowed ×7250.

walls, inside the annular collar (Fig. 29). The internal structure surrounding the aperture is composed of small pieces of quartz, hence the irregular opening. This inner apertural opening is covered by a smooth organic membrane in encysted specimens, but in some empty shells the recess of the collar is filled with small pieces of extraneous debris.

Range of measurements in μm (6 specimens examined): shell length 119–146, breadth 98–106, diameter of collar 33–49, length of collar 13–19, diameter of aperture 14–21.

In the original description (Penard, 1902) the collar was described as being four-lobed, and this was emended by Cash *et al.* (1919) to include both three and four-lobed specimens. In subsequent works (Gauthier-Lièvre & Thomas, 1960; Vucetich, 1973) the three-lobed specimens have been considered to represent a new variety and form, *C. mespiliformis* var, *africana* fma *triloba* Gauthier-Lièvre & Thomas, 1960. It is thought that this variation alone is insufficient to warrant specific identification and that these reports are best considered as *C. mespiliformis*.

The only previous British record of this species is from a pond at Husthwaite near York (Cash *et al.*, 1919), but it has been reported from Europe (Penard, 1902; Deflandre, 1927; Chardez, 1956; Thomas, 1954), America (Edmonson, 1912), South America (Vucetich, 1973; Boltovsky & Lena, 1974), and Africa (Decloitre, 1948; Gauthier-Lièvre & Thomas, 1960).

Discussion

In previous studies on pyriform specimens of *Difflugia* (Ogden, 1979*a*; 1980; Ogden & Fairman, 1979) it has been shown that size, shape and detailed shell structure can be used successfully to distinguish species. The application of these criteria as applied to five ovoid species are summarized below.

Whereas in the earlier work the shape and size of the aperture, was with few exceptions, a relatively uniform character, in ovoid forms this feature appears to be more variable. For example, although the aperture in specimens of *D. lobostoma* described here (p. 126) is generally trilobed with only a few specimens having ill-defined lobes, Penard's (1902) specimens were predominately four-lobed. It is possible that Penard's specimens represent a distinct species, but the aperture apart, his description is in good agreement with that for *D. lobostoma*.

The presence or absence of an apertural collar is another questionable diagnostic character. It is generally accepted that in most testate amoebae the aperture is formed initially during division to produce a daughter shell, and that the appearance of the remainder of the shell is accompanied by cytoplasmic movement between the parent and the newly-formed daughter. An effective junction between the two opposed apertures is therefore essential. As the apertural opening in both *D. gramen* and *D. lobostoma* is irregular, it is suggested that the small ridge of particles that borders the aperture is constructed in such a way so that it forms an effective seal between the parent and daughter shells during division in these animals. This would account for the uneven distribution of these particles around the apertural opening, and for the way in which they are often concentrated together to fill depressions or conversely to form projections. It follows that these ridges would not be identical, even between parent and daughter. In fact they vary considerably and their only real value as a diagnostic character is probably their presence or absence. This theory regarding the apertural ridges can only be tested by opposing the apertures of several parent and daughter shells, to establish whether or not such related couples match.

Although the ring of pores just posterior to the aperture in *D. gramen* is used here as a diagnostic character, being incomplete in *D. achlora* and absent in *D. lobostoma*, the function of this feature is unknown and must be used with some reservation. Its appearance in the same position in both *D. tuberculata* and *D. wailesi* certainly rules out any suggestion that it is an artifact.

The measurements given for individual specimens of *D. gramen* and *D. lobostoma* from Norfolk, would suggest that they can be separated using these parameters (see Table 1). If these measurements are presented in graphical form however, it would appear that in length and breadth, at least, there is a similarity between the two species that suggests a correlation. The slope of a line fitted by eye to all the data, would also seem to fit the data if it was treated as two separate parts. This similarity is also shown in Table 1 by comparing the ratio B/L (breadth ÷ length). The diameter of the aperture is included in the graph and as a ratio (see Table 1), but these results do not appear to be significantly different.

Stepánek & Jiri (1958) measured a hundred specimens of both *D. achlora* and *D. gramen*, and suggested that the shells of these two species appeared to be significantly different in length and breadth (see Tables 1 & 2). If the individual measurements are plotted (Fig. 31), a similar correlation between length and breadth can be shown between these two species to that illustrated for *D. gramen* and *D. lobostoma* (Fig. 7).

Table 2 Mean values (in μm)

	Length	Breadth	Diameter of aperture
D. achlora	(60·8)	(47·2)	(21·4)
D. gramen	98·8	94·3	33·6
	(81·6)	(60·4)	(25·4)
D. lobostoma	158·1	137·3	45·7

Bracketed measurements are taken from Stepánek & Jiri (1958).

By plotting the data for breadth and length in this way, it could be inferred that specimens of *D. achlora*, *D. gramen* and *D. lobostoma* exhibit progressive steps in size that possibly represent one species. Variability in the recorded measurements of *D. gramen* and *D. lobostoma* have already been mentioned (p. 126). Such differences illustrate how difficult it is to separate three similar species on size alone, and suggests that the incorrect specific designation has probably been given on several occasions. Furthermore, the suggestion that *D. achlora* and *D. gramen* could be distinguished by morphometric and statistical methods (Stepánek & Jiri, 1958), could also be interpreted as a way of differentiating between two populations of the same species, especially as the specimens of each species came from two different sources.

The present work does not help to resolve this problem, and measurements are used here to distinguish between the species. Nevertheless, I suggest that in future identifications of these three species, details of individual specimen measurements may possibly benefit the eventual solution of this species-complex. To summarize, *D. achlora*, *D. gramen* and *D. lobostoma* can be identified by size and detailed knowledge of the shell structure (i.e. number of pores surrounding the aperture and the size of the mesh in the organic network).

In the other ovoid species described here, *D. tuberculata* and *D. wailesi*, the evidence put forward to distinguish between them is also based on differences of shell structure. Specimens of *D. tuberculata* have the typical protuberances which are usually pronounced or sometimes slightly flattened, but in either case they are frequently represented by aggregates of small particles. Such aggregates are present in specimens constructed of either quartz particles or diatom frustules. *D. wailesi* by contrast has a smooth shell composed mainly of diatom frustules, with the shell components arranged irregularly, and there are no aggregates of small particles. Whilst there is a slight difference between the two species in the number of indentations surrounding the apertural opening, no particular significance is

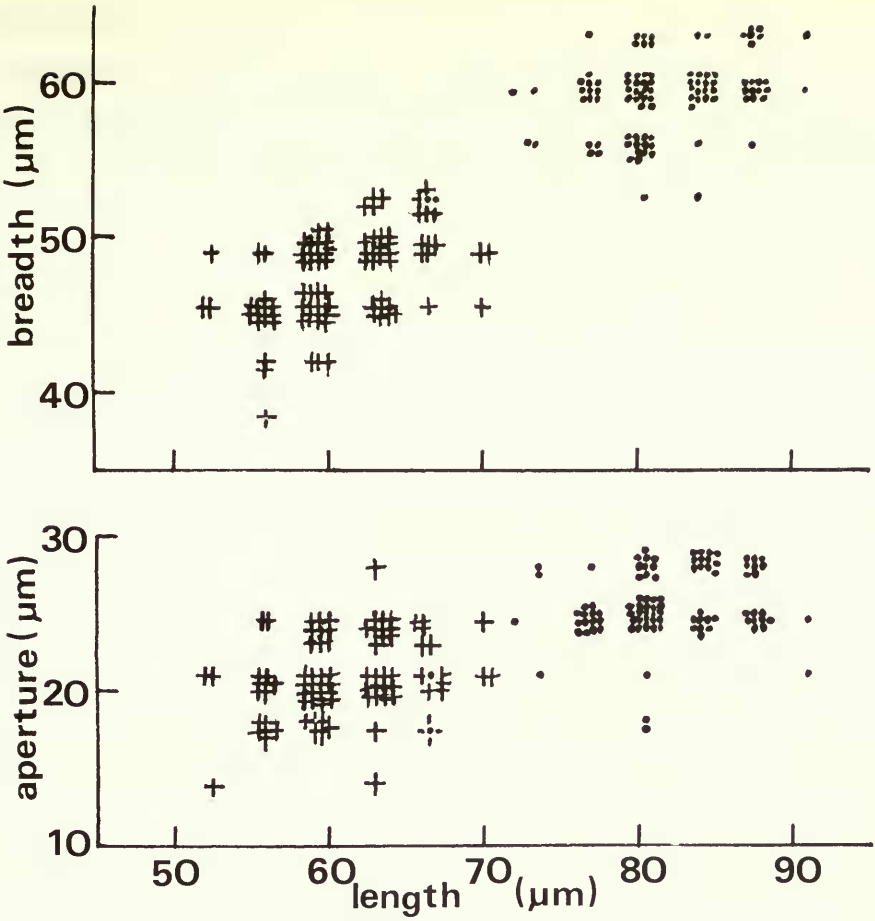


Fig. 31 Plot of individual measurements for specimens of *Difflugia achlora* (crosses) and *D. gramen* (points) taken from Stepánek & Jiri (1958)—breadth (upper) and diameter of aperture (lower) vertical axis; total shell length, horizontal axis.

attached to this feature at present, especially as the depth of the indentations varies in each specimen which suggests that this may be an unreliable character. There are slight differences in size between the two species, but the data is too sparse to be useful.

Our observations on pyriform species of *Difflugia* show that the structure and appearance of the shell have so far been reliable diagnostic characters. The ability of *D. tuberculata* to construct a similar shell of either quartz or diatom frustules would seem to strengthen this argument. Especially as there is no similar evidence of this ability in pyriform species, which tend to be more conservative in their choice of construction material. I therefore consider that the difference between the shells of *D. tuberculata* and *D. wailesi* is a sufficiently strong character to distinguish between these two ovoid species.

In conclusion, it is fair to say that the examination of these five ovoid species of *Difflugia* has shown how difficult it is to establish good diagnostic characters in these forms than it is in pyriform species.

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